8. Bürkle, L., Cedzich, A., Döpke, C., Stransky, H., Okumoto, S., Gillissen, B., Kühn, C., and Frommer, W.B. (2003). Transport of cytokinins mediated by purine transporters of the PUP family expressed in phloem, hydathodes, and pollen of *Arabidopsis*. Plant J. *34*, 13–26.
9. Gillissen, B., Bürkle, L., André, B., Kühn, C., Rentsch, D., Brandl, B., and Frommer, W.B. (2000). A new family of high-affinity transporters for adenine, cytosine, and purine derivatives in *Arabidopsis*. Plant Cell *12*, 291–300.
10. Bishopp, A., Lehesranta, S., Vatén, V., Help, H., El-Showk, E., Scheres, B., Helariutta, K., Mähönen, A.P., Sakakibara, H., and Helariutta, Y. (2011). Phloem-transported cytokinin regulates polar auxin transport and maintains vascular pattern in the root meristem. Curr. Biol. *21*, 927–932.
11. Kramer, E.M., and Bennett, M.J. (2006). Auxin transport: a field in flux. Trends Plant Sci. *11*, 382–386.
12. Friml, J., Vieten, A., Sauer, M., Weijer, D., Schwarz, H., Hamann, T., Offringa, R., and Jurgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. Nature *426*, 147–153.
13. Bishopp, A., Help, H., El-Showk, S., van den Berg, W., Weijers, D., Scheres, B., Benkova, E., Friml, J., Mahonen, A.P., and Helariutta, Y. (2011). A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. Curr. Biol. *21*, 917–926.
14. Mahonen, A.P., Bishopp, A., Higuchi, M., Nieminen, K.M., Kinoshita, K., Tormakangas, K., Ikeda, Y., Oka, A., Kakimoto, T., and Helaruitta, Y. (2006). Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. Science *311*, 94–98.
15. De Smet, I., Tetsumura, T., De Rybel, B., Frei dit Frey, N., Laplaze, L., Casimiro, I., Swarup, R., Naudts, M., Vanneste, S., Audenaert, D., *et al.* (2007). Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. Development *134*, 681–690.
16. Romanov, G.A., Lomin, S.N., and Schmulling, T. (2006). Biochemical characteristics and ligand binding properties of Arabidopsis cytokinin receptor AHK3 compared to CRE1/AHK4 as revealed by a direct binding assay. J. Exp. Bot. *57*, 4051–4058.
17. Miyawaki, K., Matsumoto-Kitano, M., and Kakimoto, T. (2004). Expression of cytokinin biosynthetic isopentyltransferase genes in Arabidopsis: tissue specificity and regulation by auxin, cytokinin and nitrate. Plant J. *37*, 28–38.

Centre for Plant Integrative Biology, University of Nottingham, LE12 5RD, UK.
E-mail: Malcolm.bennett@nottingham.ac.uk

DOI: 10.1016/j.cub.2011.04.048

Neuroanatomy: Uninhibited Connectivity in Neocortex?

The mouse neocortex is now the focus of research using twenty-first century techniques of circuit analyses, which are revealing different wiring strategies for excitatory and inhibitory connections and providing important insights into the possible computations of cortical circuits.

Kevan A.C. Martin

"The connectivity diagram of neocortical circuits is still unknown…"

With this opening statement of their report on 'Dense inhibitory connections in neocortex', Fino and Yuste [1] pitch their readers into icy water. Surely they cannot be serious that after 121 years of research on the fine structure of cortical neurons and their connections [2], we are still so far from dry land? It seems they are serious, for they finish their first sentence with the equally bone-chilling assertion that we cannot even be sure whether cortical circuits are wired specifically or randomly.

This preamble is their justification for a conceptually simple experiment in which they mapped the distribution of somatostatin-expressing neurons that inhibit single pyramidal cells in

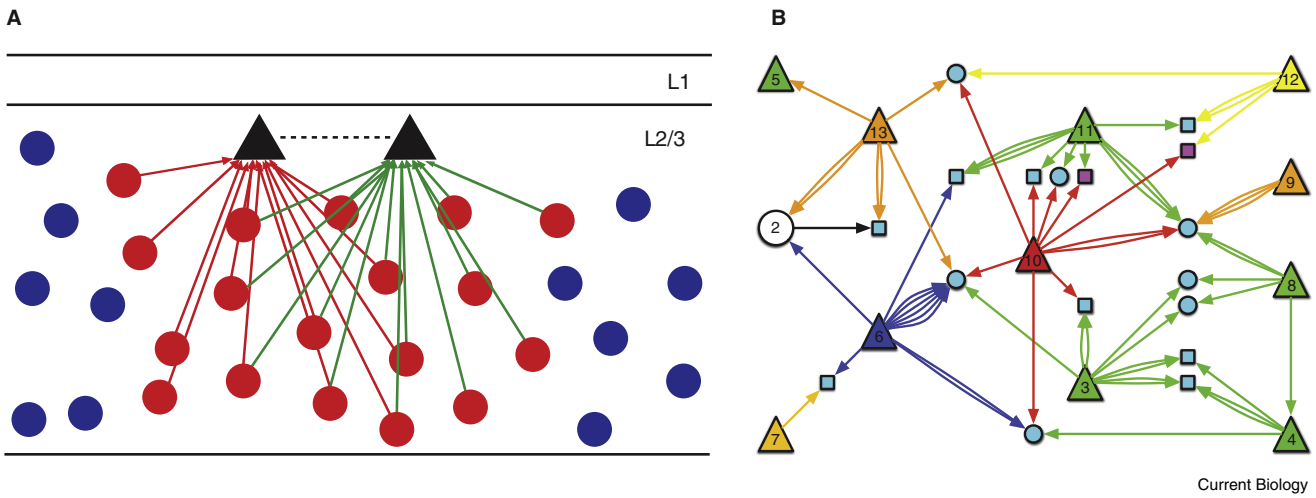

Figure 1. Models of cortical connectivity.

(A) Hypothetical circuit model showing a dense connectivity of inhibitory inputs from somatostatin-expressing GFP inhibitory neurons (filled circles) to pyramidal cells (black triangles) in cortical layer 2/3 (L2/3). Within a local region, the connectivity may be complete (connections from red circles). The dashed line between the two pyramidal cells illustrates that the same model applies whether or not the pyramidal cells are also connected with each other [1]. Adapted with permission from [1]. (B) Directed network graph of the functionally characterized cells and their targets established by Bock *et al.* [3]. Presynaptic pyramidal cells indicated as triangles, coloured according to their varied orientation preferences. Smooth (inhibitory) neuron indicated as open circle. Postsynaptic excitatory (magenta) and inhibitory (cyan) targets with cell bodies contained within the EM volume are drawn as circles. Other postsynaptic targets (dendritic fragments) are drawn as squares. Adapted with permission from [1].

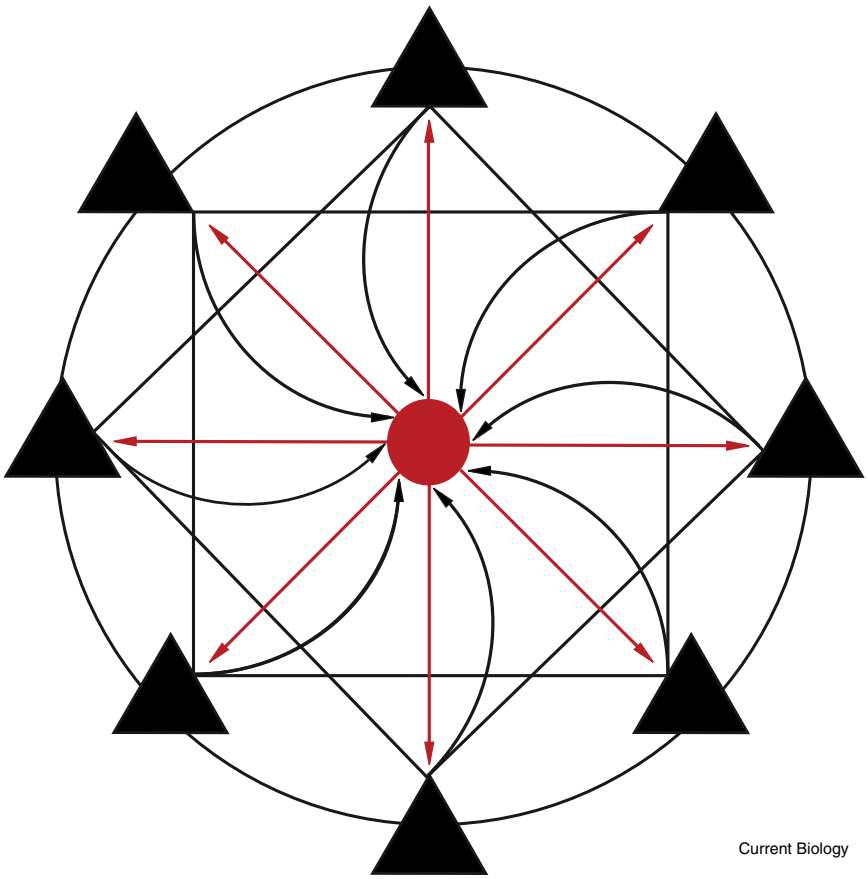

Figure 2. The 'ring-of-neurons' model of recurrent cortical circuits that perform a variety of computational operations, including 'Winner-Take-All' [6–8].

Single or pools of excitatory pyramidal cells (black triangles) are recurrently connected (curved black lines) with their neighbours and with a pool of inhibitory neurons (red filled circle, inhibitory connections indicated by red lines). A given parameter, such as orientation preference, is mapped around the circle of excitatory neurons so that nearest neighbours who lie closer together in the parameter space have similar preferences and are more strongly connected than more distant neurons in the map. Some excitatory connections (straight black lines) may skip nearest neighbors and connect to neurons with dissimilar functional preferences.

the superficial layers of the frontal cortex of the mouse. By this they hoped to determine the functional structure of one inhibitory 'network' in the neocortex. Their conclusion, in one-sentence, is that most or all of the somatostatin-expressing neurons in the vicinity of a given pyramidal cell connect to it (Figure 1A). But does their observation help us in divining the nature of the connectivity diagram of the neocortex and the rules that form it, or does it just add another byte of puzzling data to a 121-year heap?

Many variants of Fino and Yuste's [1] experiments have been performed over the past 121 years and, while the technology has changed somewhat over time, the central question has not. Since Ramon y Cajal [2] began using Golgi's eponymous stain, the constant question has been: who connects to whom? To make their discovery, Fino and Yuste [1] combined a number of fancy new technologies — a mouse strain in which somatostatin neurons were labelled with green fluorescent protein ('sGFP' neurons), two-photon microscopy, whole cell patch recordings, and a new 'caged' glutamate compound, which they used to excite selectively single sGFP neurons in slices of the frontal cortex. What they may also know, but did not tell us, is whether the pyramidal cells made reciprocal connections with the sGFP neurons.

Evidence of convergent pyramidal cell connections with smooth (inhibitory) neurons comes instead from the other end of the mouse's brain, the visual cortex. Like Fino and Yuste [1], Bock *et al.* [3] used two-photon microscopy, but now to record the calcium transients in neurons rather than stimulate the neurons. Their recordings *in vivo* provided them with a single-cell resolution map of the orientation preference of the neurons, but what they really wanted to know was how the neurons they imaged *in vivo* were wired together. The approach they chose was brute force: to reconstruct a sample of 14 imaged neurons at synaptic resolution using serial-section electron microscopy (SSEM). In what could only have been a labour of blind love, they traced *manually* the axons and dendrites of the 14 neurons through over a thousand serial sections and discovered that 13 were pyramidal cells and one was a smooth neuron — one of the inhibitory neurons of the cortex. They also mapped the targets of the axons and their significant finding was that, in a number of instances, two or more of the pyramidal cells of different orientation preferences provided excitatory input to the same inhibitory neuron (Figure 1B). Because the axons of the inhibitory neurons were not reconstructed, they could not establish whether any of these connections were reciprocal.

In a closely related study, Ko *et al.* [4] also used two-photon calcium imaging of mouse visual cortex, but they used *in vitro* electrophysiology instead of SSEM to determine the synaptic connections. Their conclusion was that connections between neighbouring pyramidal neurons are not random, but selective, because the probability of two neurons connecting increased with the degree of similarity of their responses to visual stimuli. These studies [1,3,4] all show that the local connectivity of the pyramidal cells with each other is very sparse in comparison to the dense connections to pyramidal cells of the sGFP inhibitory neurons reported by Fino and Yuste [1], and to the convergent pyramidal cell to inhibitory neuron connections traced by Bock *et al.* [3].

All three studies [1,3,4] impress with their technical wizardry, but their question is still Ramon y Cajal's [2], so it is reasonable to ask whether they — and many others employing these new technologies — are doing any better than he did with Golgi's stain and an optical microscope? What new revelations emerge about the principles of cortical wiring? Ko *et al.* [4] interpret their findings quite conventionally: cells that fire together, wire together. More surprisingly, Fino and Yuste [1] raise the old ghost of a *tabula rasa* brain formed initially

of random connections as one possible explanation for their finding. But to suppose that the sophisticated neural functions that are needed even before birth arise magically by experience-dependent re-organization of an initial network of random connections flies in the face of evidence and of logic. *Tabula rasa* has not been a serious hypothesis since Ramon y Cajal first observed that nervous systems consist of neurons that form specific connections from the earliest outgrowth of their axons and dendrites. The studies of Bock *et al.* [3] and Ko *et al.* [4] support his notion of specificity. The ghost of *tabula rasa* should be laid to rest for once and for all.

It is certainly not a general property of all inhibitory cell types that they connect to all excitatory cell targets in their vicinity or *vice versa* [5]. Thus, the densely connected inhibitory neurons observed in two of the studies reviewed here should not be taken as evidence of 'promiscuous innervation' as Fino and Yuste [1] describe it, but of a deliberate wiring strategy. What we do not yet understand is why the wiring is like it is. Fino and Yuste [1] speculate that their inhibitory circuit might perform the housekeeping function of keeping excitation within some operating range. Taken together, however, the three studies reveal circuits with much richer possibilities for computation. For example, the patterns of connections reported are consistent with the 'ring-of-neurons' model (Figure 2), which can generate a number of critical computational 'primitives' [6], including the 'soft' Winner-Take-All operation implicated in such key cortical operations as selective amplification, signal restoration, and decision-making (see, for example, [6–8]).

Then there is the matter of the 'unknown' connectivity diagram. While it is true that Ramon y Cajal [2] failed to describe a connectivity diagram for neocortex, it is also true that since the 1970s at least there have been any number of diagrams that, despite being drawn from different cortical areas and different species, show such family resemblances that we have suggested these might be 'canonical' circuits for neocortex [5]. Our hope is that these new tools will provide the means of exploring many more cortical areas in detail, rather than the one or two that dominate current studies.

The twenty-first century has given us something that the Golgi technique never could — quantitative connection maps. To the question, who connects to whom, we can now add, 'and how much?' The current enthusiasm for connectivity diagrams is clearly infectious, but these diagrams are only a necessary, not sufficient, condition for understanding the principles organising cortical circuits and their function. As Horace Barlow elegantly put it [9]: "We badly need all possible information on what one might call 'principles and technology of neural engineering' and the only way to acquire it is to relate anatomical structures and cellular function to overall performance." The technologies on display here are convincing evidence that we have never been better equipped to discover these principles.

References

1. Fino, E., and Yuste, R. (2011). Dense inhibitory connectivity in neocortex. Neuron *69*, 1188–1203.
2. DeFelipe, J., and Jones, E.G. (1988). Cajal on the Cerebral Cortex (New York: Oxford University Press), pp. 654.
3. Bock, D.D., Lee, W.-C.A., Kerlin, A.M., Andermann, M.L., Hood, G., Wetzel, A.W., Yurgenson, S., Soucy, E.R., Kim, H.S., and Reid, R.C. (2011). Network anatomy and *in vivo* physiology of visual cortical neurons. Nature *471*, 177–182.
4. Ko, H., Hofer, S.B., Pichler, B., Buchanan, K.A., Sjöström, P.J., and Mrsic-Flogel, T.D. (2011). Functional specificity of local synaptic connections in neocortical networks. Nature *473*, 87–91.
5. Douglas, R.J., and Martin, K.A.C. (2004). Neuronal circuits of the neocortex. Annu. Rev. Neurosci. *27*, 419–451.
6. Douglas, R.J., and Martin, K.A.C. (2007). Recurrent neuronal circuits in the neocortex. Curr. Biol. *17*, R496–R500.
7. Douglas, R.J., Koch, C., Mahowald, M., Martin, K.A.C., and Suarez, H.H. (1995). Recurrent excitation in neocortical circuits. Science *269*, 981–985.
8. Rutishauser, U., Douglas, R.J., and Slotine, J.J. (2011). Collective stability of networks of winner-take-all circuits. Neural Comp. *23*, 735–773.
9. Barlow, H.B. (1977). Performance, perception, dark-light, and gain boxes. In Neuronal Mechanisms in Visual Perception, E. Pöppel, R. Held, and J.E. Dowling, eds. (Cambridge: Neurosciences Research Program Bulletin 15, MIT Press), (1977) pp. 394–397.

Institute of Neuroinformatics, UZH/ETH, Winterthurerstrasse 190, 8057 Zurich, Switzerland.
E-mail: kevan@ini.phys.ethz.ch

DOI: 10.1016/j.cub.2011.04.026

Force Generation: ATP-Powered Proteasomes Pull the Rope

Recently, single-molecule force spectroscopy techniques have provided unprecedented opportunities to apply and to quantify forces that guide protein (un-)folding. A new study provides fascinating insights into the sophisticated mechanism by which an ATP-fueled proteolytic machine generates mechanical forces to unfold and translocate multidomain substrates.

Yves F. Dufrêne[1] and Daniel J. Müller[2]

The force-induced conformational changes of cellular proteins play major roles in mediating physiological functions. Prominent examples are mechanosensors, which convert mechanical forces into biochemical signals, cell-adhesion proteins such as integrins, which strengthen and regulate cell adhesion through force-induced functional states, and proteases such as ATP-dependent proteolytic machines, which recognize, unfold and translocate specific protein substrates. In a recent issue of *Cell*, Aubin-Tam *et al.* [1] use single-molecule force spectroscopy (SMFS) to study how the ClpXP protease generates force to unfold and translocate multidomain substrates.

The past several years have witnessed remarkable progress in applying SMFS to measure the molecular force response of proteins [2–6]. SMFS describes a family of related techniques that apply and measure forces over different length ($\approx$0.1 nm to 100 μm) and/or time ($\approx$0.1 μs to 100 s) scales. SMFS-based assays measure forces exerted on — and generated by — single molecules over a wide range of forces (from $\approx$0.1 pN to